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Immunogenicity of seasonal inactivated influenza and inactivated polio vaccines among children in Senegal: Results from a cluster-randomized trial

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ABSTRACT

Data on influenza vaccine immunogenicity in children are limited from tropical developing countries. We recently reported significant, moderate effectiveness of a trivalent inactivated influenza vaccine (IIV) in a controlled, cluster-randomized trial in children in rural Senegal during 2009, a year of H3N2 vaccine mismatch (NCT00893906). We report immunogenicity of IIV3 and inactivated polio vaccine (IPV) from that trial. We evaluated hemagglutination inhibition (HAI) and polio antibody titers in response to vaccination of three age groups (6 through 35 months, 3 through 5 years, and 6 through 8 years). As all children were IIV naïve, each received two vaccine doses, although titers were assessed after only the first dose for subjects aged 6 through 8 years. Seroconversion rates (4-fold titer rise or increase from <1:10 to ≥1:40) were 74–87% for A/H1N1, 76–87% for A/H3N2, and 54–79% for B/Yamagata. Seroprotection rates (HAI titer ≥ 1:40) were 79–88% for A/H1N1, 88–96% for A/H3N2, and 52–74% for B/Yamagata. IIV responses were lowest in the youngest age group, and they were comparable between ages 3 through 5 years after two doses and 6 through 8 years after one dose. We found that baseline seropositivity (HAI titer ≥ 1:10) was an effect modifier of IIV response. Using a seroprotective titer (HAI titer ≥ 1:160) recommended for IIV evaluation in children, we found that among subjects who were seropositive at baseline, 69% achieved seroprotection for both A/H1N1 and A/H3N2, while among those who were seronegative at baseline, seroprotection was achieved in 11% for A/H1N1 and 22% for A/H3N2. The IPV group had high baseline polio antibody seropositivity and appropriate responses to vaccination. Our data emphasize the importance of a two-dose IIV3 series in vaccine naïve children. IIV and IPV vaccines were immunogenic in Senegalese children.

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1. Introduction

The greatest burden of influenza disease is experienced by low- and middle-income countries (LMICs) [1–4]. Most Sub-Saharan African countries do not have national influenza vaccine programs [5], and the use of influenza vaccines is minimal in the region [6]. Optimal immunization strategies are needed for the region that would minimize costs and maximize vaccine impact and program feasibility. Vaccine programs that target school-aged children can

Abbreviations: CDC, United States Centers for Disease Control and Prevention; FDA, United States Food and Drug Administration; HAI, hemagglutination inhibition; HDSS, Health and Demographic Surveillance System; IIV3, trivalent inactivated influenza vaccine; IPV, inactivated poliovirus vaccine; P1, poliovirus type 1; P2, poliovirus type 2; P3, poliovirus type 3; TCID50, tissue culture infective dose; WHO, World Health Organization.

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decrease community-wide virus transmission and result in decreased influenza illness in other age groups [7–9]. Modeling studies in the United States suggest that targeting children (and young adults) for vaccination to reduce community transmission can have the greatest impact on population-wide influenza morbidity and mortality for low-to-moderate effectiveness vaccines as compared to other immunization strategies [10].

To better understand individual- and population-level performance of influenza vaccines in Senegal, we performed a double-blind, cluster-randomized trial comparing trivalent inactivated influenza vaccine (IIV3) with an inactivated polio vaccine (IPV) control among children aged 6 months through 10 years. We previously reported a total vaccine effectiveness of 43.6% (95% CI 18.6–60.9%) in preventing laboratory-confirmed, symptomatic influenza caused by the circulating, drifted A/H3N2 strain among age-eligible children during year one of the trial [4]. A secondary objective of the trial was to determine the immunogenicity of the study vaccines, to help understand efficacy measures. As the manufacturer-recommended two doses for influenza vaccine naïve children is programmatically more difficult for older children, we included an assessment of immunogenicity after a single-dose vaccination in children 6–8 years of age. In this report we describe the per-protocol antibody immune responses to IIV3 and IPV in a subset of children enrolled in the larger cluster-randomized clinical trial.

2. Material and methods

This study was conducted in 2009–2010 within 20 geographically contiguous villages in the Niakhar, Senegal Health and Demographic Surveillance System (HDSS) [11]. Oral polio vaccine was part of routine childhood immunization, but neither IPV nor IIV3 had been used routinely at the time of the study. The full study design was described previously [4]. In brief, healthy children 6 months through 10 years of age were eligible for study vaccination if a parent's primary residence was the Niakhar HDSS, the child's family was not expecting to migrate out of the area during the study period, and a parent was willing to provide written informed consent [12]. Children with a history of hypersensitivity to any component in either IIV3 or IPV were excluded and children with acute febrile illness (≥38 °C axillary) at the time of screening were temporarily excluded until the illness resolved.

The study products were the 2008–2009 Northern Hemisphere formulation of IIV3 (Vaxigrip, Sanofi Pasteur, Lyon, France; lots D5813 and D9672), containing A/Brisbane/59/2007 (H1N1)-like, A/Brisbane/10/2007 (H3N2)-like, and B/Florida/04/2006 (Yamagata lineage)-like strains [13], and IPV (IMOVAX Polio, Sanofi Pasteur, Lyon, France; lot B0283). Vaccines were masked before delivery to the Niakhar HDSS, and both administering nurses and personnel conducting follow-up were blinded to vaccine arm. Children aged 6 through 35 months received 2 doses of 0.25 mL IIV3 or 0.5 mL IPV intramuscularly, with 1 dose at enrollment and another dose 1 month later. Children aged 3 through 8 years received 2 doses of 0.5 mL IIV3 or 0.5 mL IPV intramuscularly [4].

2.1. Procedures

A subset of parents were consented for their children to participate in an immunogenicity substudy from two of the 20 villages, Diohine and Toucar, which were randomized to receive different study vaccines. Study personnel in Senegal were blinded to the village assignment of study vaccines, except for the unblinded vaccine manager and J.C. Victor at PATH. We aimed to collect serum at baseline (day 0, immediately prior to first vaccination) from 400 children residing in these two villages, selected evenly from 4 different age groups: 6 through 11 months, 12 through 35 months, 3 through 5 years, and 6 through 8 years of age. After study initiation, difficulty in enrolling enough children aged 6 through 11 months to participate in blood draws (only 5 children enrolled from each of the two villages) resulted in combining that age group with children aged 12 through 35 months. For the youngest two age groups, children aged 6 months through 5 years, the post vaccination serum sample was collected one month after the second dose of the two-dose course of vaccination (e.g. at day 60). We collected the post vaccination serum sample for children aged 6 through 8 years after only a single dose of the two-dose course (e.g. at day 30). Sera were drawn at this time point to assess the potential for a programmatically simpler, 1-dose influenza vaccine schedule in vaccine naïve children in children aged 6 through 8 years.

2.2. Detection of serum antibody to influenza virus by HAI

Protocol-defined endpoints included the post-vaccination serologic titer of antibodies to each strain contained in the IIV3. Titers were determined by the hemagglutination inhibition (HAI) assay using Turkey red blood cells and vaccine-matched antigens, including ether-treated influenza B virus [14]. The Pasteur Institute, Dakar, Senegal, performed the HAI testing. The immunogenicity analysis was conducted on the per-protocol population, including subjects who met inclusion criteria and received appropriate doses of study vaccines within the planned time (between 23 May and 11 July 2009). Children without both pre- and post-vaccination samples were included in baseline seropositivity analyses or post-vaccination titer analyses but were not included in fold-change analyses.

The standard regulatory definition of seroprotection (HAI titer of ≥1:40 post-vaccination) was used for the primary analysis. Seroconversion was defined as either at least a 4-fold rise in titer or an increase of HAI titer from <1:10 to ≥1:40 [15]. Stratification of immunogenicity results by baseline serostatus (with seronegative defined as HAI titer < 1:10 and seropositive defined as HAI titer ≥ 1:10) was conducted post-hoc, as was an analysis using an alternative definition of seroprotection that has been proposed for children (HAI titer ≥ 1:160 post-vaccination) [16–18].

We calculated the proportions with exact 95% confidence intervals (CIs) based on the binomial distribution and geometric mean titers (GMTs) with approximate 95% CIs for the HAI titers based on the normal distribution after log transformation. We used Graphpad Prism 8.2 (GraphPad Software, La Jolla California USA) for statistical analyses.

2.3. Serum antibody to poliovirus detected by microneutralization assay

Poliovirus vaccine immunogenicity was another protocol defined secondary outcome. Serum specimens were tested at the Pasteur Institute for the presence of antibodies to poliovirus using a microneutralization test based on the World Health Organization (WHO) standard procedure [19]. Antibody titer is expressed as the inverse of the highest serum dilution that protects 50% of cell-cultures against 100 $TCID_{50}$ of the Sabin reference viruses, serotypes 1–3 (P1, P2, P3). We defined poliovirus seropositivity as the reciprocal titer of poliovirus neutralizing antibodies ≥1:8, with the upper limit of detection 1:256 and higher titers recorded as 1:512. We further characterized the response to vaccine as seroconversion from seronegative to seropositive or 4-fold increase in the level of neutralizing antibodies after immunization.

2.4. Oversight and registration

The National Ethics Committee for Health Research (Senegal Ministry of Health and Social Welfare) and Western Institutional Review Board provided human subjects research approvals for this study. We conducted the study in accordance with the principles of the Declaration of Helsinki (2008) and in compliance with Good Clinical Practice guidelines. This study is registered with ClinicalTrials.gov, number NCT00893906.

3. Results

3.1. Study population

From the 7766 children enrolled, randomized, and administered either IIV3 or IPV between May 23 and July 11, 2009, a total of 276 children were enrolled in the immunogenicity substudy: 105 were aged 6 through 35 months, 97 were aged 3 through 5 years, and 74 were aged 6 through 8 years. Of these 276 children, 218 (79%) with at least one serum sample and who met other per-protocol analysis entry criteria were analyzed; 108 received IIV3 and 110 received IPV (Table 1).

3.2. Baseline seropositivity

Pre-vaccination titers were similar between the two villages. Both arms demonstrated a high proportion of subjects with seropositivity (HAI titer > 1:10) to the influenza A strains included in the IIV3 vaccine. In the older age groups, this seropositivity was as high as 87% for A/H1N1 and 92% for A/H3N2 (Fig. 1). In contrast, 40% or fewer children in any age group were seropositive to influenza B at baseline. The youngest age group (ages 6 through 35 months) had lower baseline seropositivity than the older two age groups for each of the three strains. Of the 10 children under 12 months of age, none had HAI titers demonstrating seroprotection (HAI $\geq$ 1:40) against any of the three vaccine strains at baseline (Supplemental Fig. 1).

Table 1
Description of immunogenicity study population.

Allocation	IIV3	IPV
Total	108	110
6 through 35 months	43 (40%)	31 (28%)
3 through 5 years	42 (39%)	39 (35%)
6 through 8 years	23 (21%)	40 (36%)
Male (%)	56 (52%)	54 (49%)
6 through 35 months	22 (51%)	18 (58%)
3 through 5 years	26 (62%)	14 (36%)
6 through 8 years	8 (35%)	22 (55%)

Note: IIV3 is trivalent, inactivated influenza vaccine, and IPV is inactivated polio vaccine.

3.3. Influenza antibody response to vaccination

Among children vaccinated with IIV3, seroprotective responses (HAI titer $\geq$ 1:40) to A/H3N2 were achieved in >88% of children in all age groups (Table 2). Post-vaccination seroprotection rates for the A/H1N1 vaccine antigen were 79% in the youngest age group, 88% in children aged 3 through 5 years, and 87% (after the first vaccine dose) in the oldest age group. Notably, approximately ¾ ($\geq$74%) of all three age groups demonstrated seroconversion (either 4-fold rise in titer or increase from HAI titer <1:10 to $\geq$1:40) to both influenza A strains, with the lower bound of the confidence interval above 40%. Immunogenicity against influenza B was less robust, with seroprotection rates of 52% of children 6 through 35 months, 74% in children 3 through 5 years, and 65% of children 6 through 8 years. Although appropriate seroconversion was achieved in the 3 through 5 years and 6 through 8 years age groups, only 54% of children (CI 39–68%) seroconverted to influenza B in the 6 through 35 month age group (Table 2). Because the $\geq$1:40 titer as a marker of seroprotection in children has been challenged as too low to reflect seroprotection, we also assessed rates of children achieving a higher titer using $\geq$1:160 as a threshold, a 2-fold dilution higher than the standard definition [16–18]. Using our higher threshold of seroprotection, only 60% of children (all age groups) had a HAI titer of 1:160 or greater against A/H1N1, 64% achieved the same titer against A/H3N2, and 30% against influenza B.

For all strains, increased age and pre-existing seropositivity were associated with higher post-vaccination GMTs. Children aged 6 through 8 years achieved 95% seroprotection (CI 79–99%) to A/H3N2 after one vaccine dose and achieved a GMT of 459 (95% CI 256–825), comparable to the 93% seroprotection (CI 81–98) and GMT of 331 (213–513) after two vaccine doses in aged 3

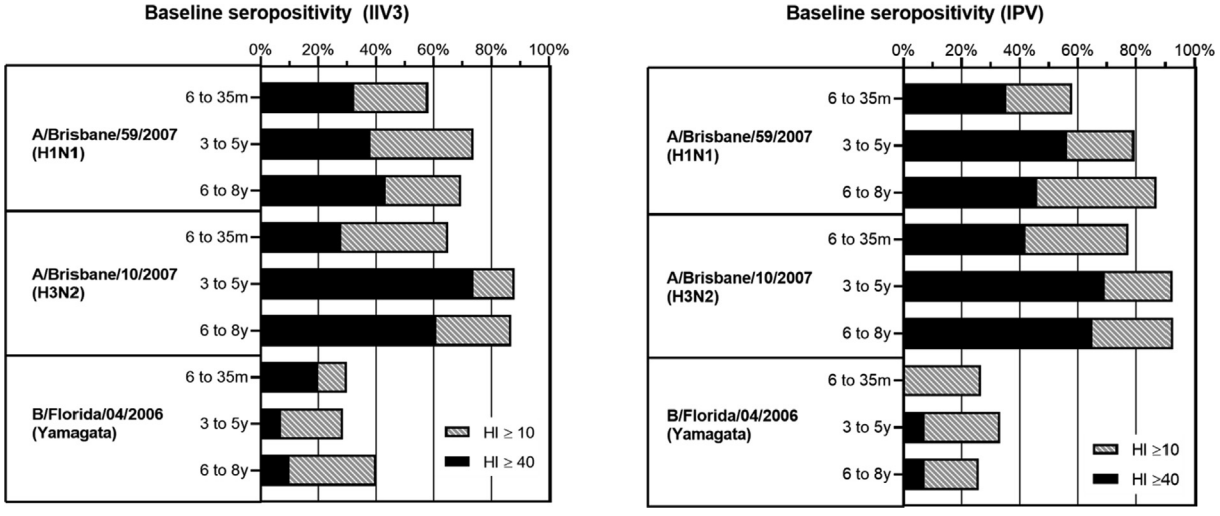

Fig. 1. Baseline seropositivity to influenza strains included in the 2008–2009 trivalent inactivated influenza (IIV3) vaccine by age groups and vaccine assignment. Note: Seropositivity is shown as percentage of children with hemagglutinin inhibition (HAI) titers $\geq$10, and with the solid portion indicating percentage of children with HAI titers $\geq$40 (a common measure of seroprotection).

Table 2
Hemagglutination inhibition titers after vaccination stratified by vaccine, age group, and influenza strain[1].

Allocation:		**IIV3**			**IPV**		
Doses before sample collection:		(0.25 mL ×2)	(0.5 mL ×2)	(0.5 mL ×1)	(0.5 mL ×2)	(0.5 mL ×2)	(0.5 mL ×2)
Age Group:		**6–35 months**	**3–5 years**	**6–8 years**	**6–35 months**	**3–5 years**	**6–8 years**
A/Brisbane/59/2007 (H1N1)							
Baseline	# analyzed	43	42	23	31	39	39
	HAI titer ≥ 40 (95% CI)	32.6% (20.5–47.5%)	38.1% (25.0–53.2%)	43.5% (25.6–63.2%)	35.5% (21.1–53.1%)	56.4% (41.0–70.7%)	46.2% (31.6–61.4%)
	HAI titer ≥ 160 (95% CI)	2.3% (0.1–12.1%)	9.5% (3.8–22.1%)	13% (4.5–32.1%)	9.7% (3.3–24.9%)	12.8% (5.6–26.7%)	10.3% (4.1–23.6%)
	GMT (95% CI)	15 (11–22)	20 (14–30)	21 (12–38)	16 (10–25)	29 (19–42)	28 (19–39)
Post-vaccination	# analyzed	43	42	23	31	39	40
	HAI titer ≥ 40 (95% CI)	79.1% (64.8–88.6%)	88.1% (75–94.8%)	87.0% (67.9–95.5%)	35.5% (21.1–53.1%)	56.4% (41–70.7%)	50.0% (35.2–64.8%)
	HAI titer ≥ 160 (95% CI)	44.2% (30.4–58.9%)	66.7% (51.6–79%)	78.3% (58.1–90.3%)	6.5% (1.1–20.7%)	15.4% (7.2–29.7%)	15% (7.1–29.1%)
	GMT (95% CI)	128 (74–220)	271 (165–447)	372 (181–766)	18 (12–29)	29 (19–43)	29 (19–44)
	Seroconversion % (95% CI)	**74.4%** (59.8–85.1%)	**76.2%** (61.5–86.5%)	**87.0%** (67.9–95.5%)	**16.1%** (7.1–32.6%)	**7.7%** (2.7–20.3%)	**9.7%** (3.3–24.9%)
A/Brisbane/10/2007 (H3N2)							
Baseline	# analyzed	43	42	23	31	39	40
	HAI Ab titer ≥ 40 (95% CI)	27.9% (16.7–42.7%)	73.8% (58.9–84.7%)	60.9% (40.8–77.8%)	41.9% (26.4–59.2%)	69.2% (53.6–81.4%)	65% (49.5–77.9%)
	HAI titer ≥ 160 (95% CI)	7% (2.4–18.6%)	14.3% (6.7–27.8%)	30.4% (15.6–50.9%)	6.5% (1.1–20.7%)	17.9% (9–32.7%)	20% (10.5–34.8%)
	GMT (95% CI)	16 (11–23)	45 (30–66)	41 (22–76)	24 (15–40)	40 (28–57)	39 (27–55)
Post-vaccination	# analyzed	43	42	23	31	39	40
	HAI Ab titer ≥ 40 (95% CI)	88.4% (75.5–94.9%)	92.9% (81.0–97.5%)	95.7% (79–99.2%)	45.2% (29.2–62.2%)	76.9% (61.7–87.4%)	60.0% (44.6–73.7%)
	HAI titer ≥ 160 (95% CI)	44.2% (30.4–58.9%)	76.2% (61.5–86.5%)	78.3% (58.1–90.3%)	12.9% (5.1–28.9%)	30.8% (18.6–46.4%)	32.5% (20.1–48%)
	GMT (95% CI)	102 (69–150)	331 (213–513)	459 (256–825)	24 (15–39)	51 (33–79)	44 (28–70)
	Seroconversion % (95% CI)	**76.7%** (62.3–86.8%)	**76.2%** (61.5–86.5%)	**87.0%** (67.9–95.5%)	**12.9%** (5.1–28.9%)	**10.3%** (4.1–23.6%)	**12.5%** (5.5–26.1%)
B/Florida/04/2006 (Yamagata lineage)							
Baseline	# analyzed	30	14	10	15	27	27
	HAI Ab titer ≥ 40 (95% CI)	20.0% (9.5–37.3%)	7.1% (0.4–31.5%)	10.0% (0.5–40.4%)	0.0% (0.0–20.4%)	7.4% (1.3–23.4%)	7.4% (1.3–23.4%)
	HAI titer ≥ 160 (95% CI)	3.3% (0.2–16.7%)	0% (0–21.5%)	10% (0.5–40.4%)	0% (0–20.4%)	0% (0–12.5%)	0% (0–12.5%)
	GMT (95% CI)	9 (6–14)	7 (5–12)	10 (4–22)	6 (5–8)	7 (6–9)	7 (5–10)
Post-vaccination	# analyzed	40	42	23	30	38	39
	HAI Ab titer ≥ 40 (95% CI)	52.5% (37.5–67.1%)	73.8% (58.9–84.7%)	65.2% (44.9–81.2%)	10.0% (3.5–25.6%)	13.2% (5.8–27.3%)	0.0% (0.0–9%)
	HAI titer ≥ 160 (95% CI)	25% (14.2–40.2%)	31% (19.1–46%)	39.1% (22.2–59.2%)	3.3% (0.2–16.7%)	0% (0–9.2%)	0% (0–9%)
	GMT (95% CI)	37 (23–61)	56 (36–86)	67 (30–147)	8 (5–11)	9 (7–12)	6 (5–7)
	Seroconversion % (95% CI)	**53.5%** (38.9–67.5%)	**78.6%** (64.1–88.3%)	**65.2%** (44.9–81.2%)	**9.7%** (3.3–24.9%)	**15.4%** (7.2–29.7%)	**2.5%** (0.1–12.9%)

1. Influenza B HAI titers initially did not include the necessary pre-treatment with ether, and on re-testing an inadequate supply of pre-treatment serum remained for some of the children. Available influenza B pre-treatment numbers are therefore smaller than for A/H1N1 or A/H3N2.

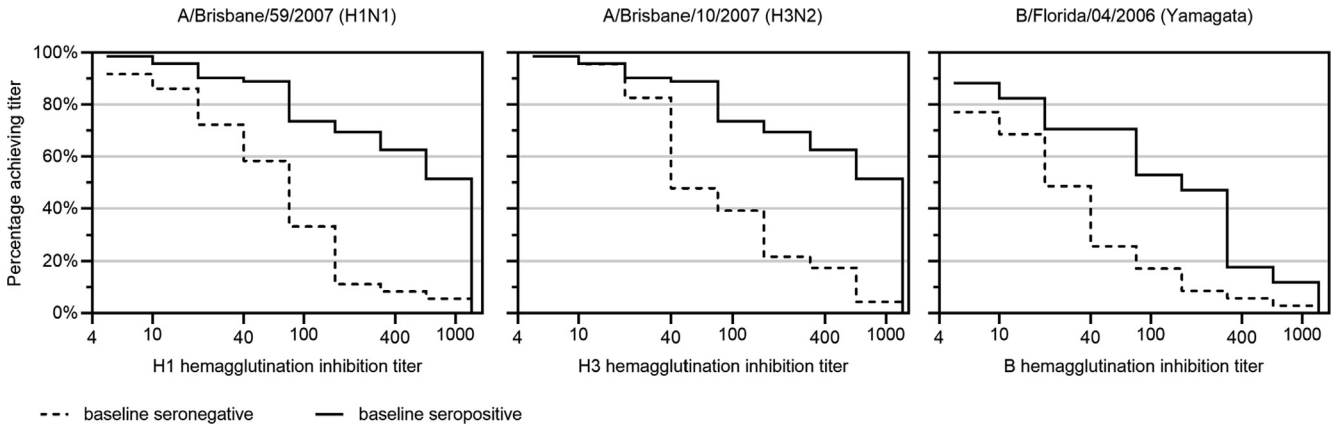

Fig. 2. Reverse cumulative distribution of HAI titers for influenza strains contained in IIV3, among all ages stratified by baseline seropositivity.

through 5 years. Seroprotection rates in children aged 6 through 8 years were less for seasonal A/H1N1 or influenza B strains than for A/H3N2 (87% with CI 68–96% for H1N1, 65% with CI 45–81% for influenza B) although the achieved titers were comparable after a single vaccine dose in this age group and children aged 3 through 5 years after two vaccine doses. For A/H1N1, the children aged 6 through 8 years had a GMT of 372 (95% CI 181–766) after as single dose compared to 271 (165–447) for ages 3 through 5 years after two doses. Using again the higher threshold of seroprotection, 69% of baseline seropositive children achieved a titer of ≥1:160 against H3N2, while only 22% of baseline seronegative children achieved the same (Fig. 2). Against seasonal A/H1N1, 69% of baseline seropositive children achieved a titer of ≥1:160, but only 11% of seronegative children achieved the same titer. For influenza B, 47% of baseline seropositive children achieved a titer ≥1:160, compared to 9% of seronegative children. Among all age groups, the A/H1N1 baseline seropositivity strongly affected post-vaccination titers, such that baseline seropositive children aged 6 through 35 months had higher post-vaccination GMTs than baseline seronegative children from either of the two older groups (Supplemental Fig. 2).

3.4. Polio antibody response to vaccination

At the time of the trial, trivalent OPV was part of the routine immunization program in Senegal, and the children receiving IPV vaccine showed high seropositivity to all three poliovirus vaccine strains at baseline. More than ninety percent of children of the older groups were baseline seropositive for P2. The vaccine was immunogenic against all strains, with mean fold changes of 1.9–3.8 for P1, 1.3–1.5 for P2, and 2.5–3.3 for P3. (Table 3). The lower fold-change associated with P2 is likely the consequence of the higher baseline seropositivity for this strain, as well as the relatively low upper limit of quantification (1:256).

4. Discussion

IIV3 immunogenicity was robust in this population of rural Senegalese children as measured by seroprotection and seroconversion rates. GMTs were higher for influenza A than B strains, as well as for older compared to younger age groups. From 80 to 96% of the youngest children developed seroprotective antibody titers in response to the A strain antigens, but only half developed a seroprotective antibody titer in response to the B strain antigen. Influenza B seroconversion in children 3–5 years was comparable to rates for influenza A strains, although seroconversion after a single dose in children aged 6–8 years was lower. These lower antibody responses to the B strain are consistent with prior studies in other populations and may reflect either diminished immunogenicity of the B strain antigens or decreased sensitivity of the HAI assay for influenza B viruses [20,21]. As we showed in our primary analysis publication [4], the low number of influenza type B infections (5 cases total between both arms) and wide efficacy estimates in this trial means it is not possible to equate the measured immunogenicity with efficacy.

While HAI titer ≥1:40 is considered the threshold defining seroprotection, corresponding with a 50% reduction in influenza virus infection in adults, higher titers may be required to provide seroprotection in children [16–18]. In the primary analysis of our Senegal trial, children under 3 years of age were the only group in which vaccine effectiveness was not demonstrated for A/H3N2: 20.6% (CI −16.3 to 45.8), compared to ~60% effectiveness for children ≥3 years of age [4]. In this immunogenicity study, we found that in this age group the GMT was 102 (95% CI 69–150) compared to >300 for children ≥3 years of age, consistent with higher titers being more protective.

Baseline influenza seropositivity was common in the study population, as indicated by HAI titers ≥1:10, suggesting prior exposure to an antigenically similar virus. In the preceding 8 years in Senegal, influenza A/H3N2 was predominant among identified circulating strains (52% of detected strains), followed by influenza A/H1N1 (23%) [22]. By the week after the second dose of study vaccinations (2009 week 28), A/H3N2 was widely circulating [4,22]. In the single year of our trial, attack rates to the circulating strain were high, from 9% in the oldest age groups to 19% in the youngest [4]. The IPV group had comparable HAI titers before and after the vaccine series, indicating that the start of transmission of A/H3N2 in the community likely did not affect immunogenicity measurements for that strain.

Programmatic feasibility is important in low resource countries, and fewer total vaccinations enhance feasibility. Thus, we evaluated the response to the first dose of vaccine in the 6–8 years group. Encouragingly, this age group had HAI titers after one dose of IIV3 that were high and comparable to the immune responses seen after two doses in the 3–5 years group. This is likely a reflection of prior exposure, as baseline seropositivity was associated with higher post-vaccination GMTs for every vaccine strain. The greatest effect was seen against A/H1N1, such that post-vaccine GMTs in the baseline seropositive youngest age group was higher than for baseline seronegative older groups (Supplemental Fig. 2). As we assessed the programmatically simpler, 1-dose IIV3 immunogenicity in the oldest age group, we did not measure responses after the second vaccine dose and cannot be sure if

Table 3
Poliovirus titers stratified by vaccine, age group, and virus strain.

Allocation:		**IIV3**			**IPV**		
Age group:		**6–35 m**	**3-5y**	**6-8y**	**6–35 m**	**3-5y**	**6-8y**
P1							
Baseline	# analyzed	35	41	22	29	36	39
	% titer ≥ 8 (95% CI)	48.6% (33–64.4%)	68.3% (53–80.4%)	90.9% (72.2–98.4%)	89.7% (73.6–96.4%)	77.8% (61.9–88.3%)	87.2% (73.3–94.4%)
	GMT (95% CI)	16.3 (7.7–34.6)	26.1 (13.9–49.2)	49.7 (23.5–105.3)	44.7 (22–91)	35.2 (19.6–63.3)	46.5 (29.8–72.6)
Post-vaccination	# analyzed	31	42	22	26	38	40
	% titer ≥ 8 (95% CI)	48.4% (32–65.2%)	64.3% (49.2–77%)	77.3% (56.6–89.9%)	88.5% (71–96%)	86.8% (72.7–94.2%)	95% (83.5–99.1%)
	GMT (95% CI)	14 (6.2–31.8)	16 (9.2–27.8)	37.5 (16.8–83.7)	93 (42.6–202.9)	130.4 (67.9–250.2)	90.5 (54.2–151)
	Mean Fold Change (95% CI)	**0.6** (0.4–1.1)	**0.6** (0.3–1.2)	**0.7** (0.3–1.6)	**2.3** (0.7–7.6)	**3.8** (1.6–9.2)	**1.9** (0.9–3.8)
P2							
Baseline	# analyzed	35	41	22	29	36	39
	% titer ≥ 8 (95% CI)	68.6% (52–81.4%)	92.7% (80.6–97.5%)	100% (85.1–100%)	93.1% (78–98.8%)	94.4% (81.9–99%)	97.4% (86.8–99.9%)
	GMT (95% CI)	39.8 (19.3–82.2)	74.5 (45.2–122.9)	90.5 (50.5–162.1)	87.3 (48.7–156.5)	107.6 (70.9–163.4)	94.6 (61.5–145.6)
Post-vaccination	# analyzed	31	41	22	26	38	40
	% titer ≥ 8 (95% CI)	61.3% (43.8–76.3%)	75.6% (60.7–86.2%)	90.9% (72.2–98.4%)	88.5% (71–96%)	92.1% (79.2–97.3%)	97.5% (87.1–99.9%)
	GMT (95% CI)	25.6 (11.1–58.9)	42.7 (21.9–83)	60.1 (28.5–126.7)	121.4 (58.5–251.8)	153.6 (88–268.2)	147 (96–225.3)
	Mean Fold Change (95% CI)	**0.5** (0.2–1)	**0.6** (0.3–1.2)	**0.6** (0.3–1.4)	**1.3** (0.6–2.8)	**1.4** (0.7–3)	**1.5** (0.8–2.8)
P3							
Baseline	# analyzed	35	41	22	29	36	39
	% titer ≥ 8 (95% CI)	57.1% (40.9–72%)	63.4% (48.1–76.4%)	81.8% (61.5–92.7%)	72.4% (54.3–85.3%)	77.8% (61.9–88.3%)	89.7% (76.4–95.9%)
	GMT (95% CI)	13.1 (6.9–24.9)	16.3 (9.6–27.7)	26.5 (13.9–50.5)	26.4 (13–53.6)	30.2 (16.7–54.6)	26.3 (17.9–38.7)
Post-vaccination	# analyzed	31	42	22	26	38	40
	% titer ≥ 8 (95% CI)	58.1% (40.8–73.6%)	71.4% (56.4–82.8%)	90.9% (72.2–98.4%)	80.8% (62.1–91.5%)	78.9% (63.7–88.9%)	90% (76.9–96%)
	GMT (95% CI)	21.4 (9.3–49.1)	25.4 (13.4–48.2)	32 (16.8–60.9)	53.1 (23.2–121.6)	97.4 (47.2–200.7)	92.1 (51.2–165.7)
	Mean Fold Change (95% CI)	**1.1** (0.5–2.5)	**1.6** (0.8–3.3)	**1.1** (0.4–3.1)	**2.5** (1–6.4)	**3.3** (1.3–8.4)	**3.3** (1.7–6.8)

further antibody increases may have occurred. However, data in US children suggest that while seronegative children benefit from the second dose as measured by improvements in HAI titers, seropositive children do not [20]. This supports the recommendation for a two dose primary series to optimize immunogenicity in vaccine naïve children, despite the associated programmatic challenges [23].

Half-dose influenza vaccines were used in children aged 6 through 35 months in accordance with manufacturer recommendations at the time of this study [13,24]. However, many countries are now recommending full dose vaccines in all children ≥6 months based on favorable reactogenicity profiles of current vaccines [25]. Full dose IIV3 vaccines may further improve immunogenicity and facilitate program implementation [26,27]. Additionally, adjuvanted vaccines have shown improved responses in younger and seronegative children [28]. Finally, increased influenza vaccine coverage in children will provide not only same-season protection but may also likely enhance immunologic responses to future influenza vaccines.

Recognizing the difficulty of comparing HAI titers across studies, our immunogenicity results are like those seen in a pediatric IIV3 study in Finland and Germany also conducted in 2009. In that study, IIV3 vaccine effectiveness was 45% (95% CI 16–64%) against vaccine matched strains and A/H3N2, compared to 44% (95% CI 19–61%) in Senegal [4,21]. Our data show comparable proportions of children achieving HAI titers ≥1:40 against homologous seasonal A/H1N1 or A/H3N2 [21]. The Niakhar HDSS includes a population similar to much of rural West Africa, with low HIV prevalence and decreasing child mortality [11]. Our immunogenicity results are encouraging, and the 2009 IIV3 studies suggest that influenza vaccines will be comparably immunogenic in diverse populations.

IPV was provided as a beneficial active comparator. From 2003 to 2009, estimated coverage by receipt of the third polio vaccine dose in Senegal ranged from 73% (2003) to 93% (2007) [29]. This high coverage is seen in the high baseline seropositivity to poliovirus strains. Although the fold-change responses are likely limited by the relatively low upper limit of detection, the post-vaccination GMTs demonstrate appropriate IPV immunogenicity in this OPV-vaccinated population. This is relevant as IPV has recently been introduced into routine immunization schedules throughout the world as part of the polio eradication effort.

There were limitations to this study. The initial protocol design included four age groups, but due to an under-enrollment of children under the age of 12 months this age group was combined with children aged 12 through 35 months for analyses. This resulted in an apparently high baseline seropositivity in the youngest age group, although individual responses showed no child <12 months of age had the ≥1:40 seroprotection titer for any vaccine strain at baseline (Supplemental Fig. 1). The pre-vaccination B/Yamagata titers were not available for all children, so seroconversion rates may have been less accurate. For IPV immunogenicity evaluations, the upper limit of detection for IPV titers was 1:256, such that IPV immunogenicity may also have been under measured.

Factors associated with influenza vaccine performance remain insufficiently studied in African countries despite the high incidence of influenza [3,30,31]. Key findings of our trial include the promising immunogenicity responses to the influenza A strains and the comparatively poor responses to influenza B, particularly in the youngest age group. Total vaccine effectiveness for the predominant seasonal influenza strain (A/H3N2) was approximately 60% for children ≥3 years, consistent with the high vaccine immunogenicity in this population [4]. Finally, the association between baseline seropositivity and increased immune response is consistent with findings in other populations and highlights

the critical need for strategies to improve influenza vaccine immunogenicity for seronegative children.

5. Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of any collaborating institution.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.09.059.

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